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(54) Title: METHODS FOR REGULATING GASTROINTESTINAL MOTILITY

(57) Abstract

Methods for reducing gastric motility and delaying gastric emptying for therapeutic and diagnostic purposes are disclosed which comprise administration of an effective amount of an exendin or an exendin agonist. Methods for treating conditions associated with elevated, inapropriate, or undesired post-prandial blood glucose levels are disclosed which comprise administration of an effective amount of an exendin or an exendin agonist alone or in conjunction with other anti-gastric emptying agents.

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METHODS FOR REGULATING GASTROINTESTINAL MOTILITY

Related Application

This application is continuation-in-part of U.S. Patent Application Serial No. 08/694,954 filed August 8, 1996, the contents of which are hereby incorporated by this reference.

Field of the Invention

The present invention relates to methods for regulating gastrointestinal motility. More particularly, the invention relates to the use of exendins and analogs and agonists thereof for the treatment of disorders which would be benefited with agents useful in delaying and/or slowing gastric emptying.

Background

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed invention, nor that any of the publications specifically or implicitly referenced are prior art to that invention.

Publications and other materials including patents and patent applications used to illuminate the specification are hereby incorporated by reference.

Exendin

The exendins are peptides that are found in the venom of the Gila-monster, a lizard found in Arizona. Exendin-3 [SEQ. ID. NO. 1] is present in the venom of

Heloderma horridum, and exendin-4 [SEQ. ID. NO. 2] is present in the venom of Heloderma suspectum (Eng, J., et al., J. Biol. Chem., 265:20259-62, 1990; Eng., J., et al., J. Biol. Chem., 267:7402-05, 1992). The exendins have some sequence similarity to several members of the glucagon-like peptide family, with the highest homology, 53%, being to GLP-1[7-36]NH_2 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993). GLP-1[7-36]NH_2 [SEQ. ID. NO. 3] is also known as proglucagon[78-107], or simply the shorthand "GLP-1," which is used interchangeably with GLP-1[7-36]NH_2 throughout this application. The sequences of exendin-3, exendin-4 and GLP-1 are shown in Figure 1. GLP-1 has an insulinotropic effect, stimulating insulin secretion from pancreatic β-cells; GLP-1 also inhibits glucagon secretion from pancreatic α-cells (Ørskov, et al., Diabetes, 42:658-61, 1993; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). GLP-1 is reported to inhibit gastric emptying (Willms B, et al., J Clin Endocrinol Metab 81 (1): 327-32, 1996; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993), and gastric acid secretion. Schjoldager BT, et al., Dig Dis Sci 34 (5): 703-8, 1989; O'Halloran DJ, et al., J Endocrinol 126 (1): 169-73, 1990; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993). GLP-1[7-37], which has an additional glycine residue at its carboxy terminus, also stimulates insulin secretion in humans (Ørskov, et al., Diabetes, 42:658-61, 1993).

A transmembrane G-protein adenylate-cyclase-coupled receptor believed to be responsible for the insulinotropic effect of GLP-1 has been cloned from a β-cell line (Thorens, Proc. Natl. Acad. Sci. USA 89:8641-45 (1992), hereinafter referred to as the "cloned GLP-1 receptor."

Exendin-4 is reportedly a potent agonist at GLP-1 receptors on insulin-secreting βTC1 cells, at dispersed acinar cells from guinea pig pancreas, and at parietal cells from stomach; the peptide is also reported to stimulate somatostatin release and inhibit gastrin release in isolated stomachs (Goke, et al., J. Biol. Chem. 268:19650-55, 1993; Schepp, et al., Eur. J. Pharmacol., 69:183-91, 1994; Eissele, et al., Life Sci., 55:629-34, 1994). Exendin-3 and exendin-4 were found to be GLP-1 agonists in stimulating cAMP production in, and amylase release from, pancreatic acinar cells (Malhotra, R., et al., Regulatory Peptides,41:149-56, 1992; Raufman, et al., J. Biol. Chem. 267:21432-37, 1992; Singh, et al., Regul. Pept. 53:47-59, 1994). Based on the insulinotropic activities of exendin-3 and exendin-4, their use has been proposed for the treatment of diabetes mellitus and the prevention of hyperglycemia (Eng, U.S. Patent No. 5,424,286).

In contrast to the full-length exendins, truncated exendin peptides such as exendin[9-39], a carboxyamidated molecule, and fragments 3-39 through 9-39 of exendin have been reported to be potent and selective antagonists of GLP-1 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993; Schepp, W., et al., Eur. J. Pharm. 269:183-91, 1994; Montrose-Rafizadeh, et al., Diabetes,45(Suppl. 2):152A, 1996). Exendin[9-39], the sequence of which is shown in Figure 1, reportedly blocks endogenous GLP-1 in vivo, resulting in reduced insulin secretion. Wang, et al., J. Clin. Invest., 95:417-21, 1995; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). Exendins and exendin[9-39] bind to the cloned GLP-1 receptor (Fehmann HC, et al., Peptides 15 (3): 453-6, 1994; Thorens B, et

al., Diabetes 42 (11): 1678-82, 1993). In cells transfected with the cloned GLP-1 receptor, exendin-4 is an agonist, i.e., it increases cAMP, while exendin[9-39] is an antagonist, i.e., it blocks the stimulatory actions of exendin-4 and GLP-1.

Exendin[9-39] is also reported to act as an antagonist of the full length exendins, inhibiting stimulation of pancreatic acinar cells by exendin 3 and exendin 4 (Raufman, et al., J. Biol. Chem. 266:2897-902, 1991; Raufman, et al., J. Biol. Chem., 266:21432-37, 1992). Exendin[9-39] is said to inhibit the stimulation of plasma insulin levels by exendin 4, and inhibits the somatostatin release-stimulating and gastrin release-inhibiting activities of exendin-4 and GLP-1 (Kolligs, F., et al., Diabetes, 44:16-19, 1995; Eissele, et al., Life Sciences, 55:629-34, 1994).

Agents which serve to delay gastric emptying have found a place in medicine as diagnostic aids in gastro-intestinal radiologic examinations. For example, glucagon is a polypeptide hormone which is produced by the α cells of the pancreatic islets of Langerhans. It is a hyperglycaemic agent which mobilizes glucose by activating hepatic glycogenolysis. It can to a lesser extent stimulate the secretion of pancreatic insulin. Glucagon is used in the treatment of insulin-induced hypoglycaemia when administration of glucose intravenously is not possible. However, as glucagon reduces the motility of the gastro-intestinal tract it is also used as a diagnostic aid in gastro-intestinal radiological examinations. Glucagon has also been used in several studies to treat various painful gastro-intestinal disorders associated with spasm. Daniel, et al. (Br. Med.

J., 1974, 3, 720) reported quicker symptomatic relief of acute diverticulitis in patients treated with glucagon compared with those who had been treated with analgesics or antispasmodics. A review by Glauser, et al., (J. Am. Coll. Emergency Physns, 8:228, 1979) described relief of acute oesophageal food obstruction following glucagon therapy. In another study glucagon significantly relieved pain and tenderness in 21 patients with biliary tract disease compared with 22 patients treated with placebo (M.J. Stower, et al., Br. J. Surg., 69:591-2, 1982).

Methods for regulating gastrointestinal motility using amylin agonists are described in International Application No. PCT/US94/10225, published March 16, 1995.

SUMMARY OF THE INVENTION

The present invention concerns the surprising discovery that exendins are potent inhibitors of gastric emptying. Exendins and exendin agonists are useful as inhibitors of gastric emptying for the treatment of, for example, diabetes mellitus, obesity, the ingestion of toxins, or for diagnostic purposes.

The present invention is directed to novel methods for reducing gastric motility and slowing gastric emptying, comprising the administration of an exendin, for example, exendin 3 [SEQ ID NO. 1], exendin 4 [SEQ ID NO. 2], or other compounds which effectively bind to the receptor at which exendins exert their action on gastric motility and gastric emptying. These methods will be useful in the treatment of, for example, post-prandial hyperglycemia, a complication associated with type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes mellitus.

In a first aspect, the invention features a method of beneficially regulating gastrointestinal motility in a subject by administering to said subject a therapeutically effective amount of an exendin or an exendin agonist. By "exendin agonist" is meant a compound which mimics the effects of exendins on gastric motility and gastric emptying, namely, a compound which effectively binds to the receptor at which exendins exert their action on gastric motility and gastric emptying, preferably an analog or derivative of an exendin.

Exendin agonist compounds useful in present invention include those compounds of the formula (I) [SEQ. ID. NO. 4]:

1 5 10
Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_4 Xaa_5 Xaa_6 Xaa_7 Xaa_8
15 20
Ser Lys Gln Xaa_9 Glu Glu Glu Ala Val Arg Leu

25 30
Xaa_{10} Xaa_{11} Xaa_{12} Xaa_{13} Leu Lys Asn Gly Gly Xaa_{14}
35
Ser Ser Gly Ala Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18}-Z

wherein Xaa_1 is His, Arg or Tyr; Xaa_2 is Ser, Gly, Ala or Thr; Xaa_3 is Asp or Glu; Xaa is Phe, Tyr or naphthalanine; Xaa_5 is Thr or Ser; Xaa_6 is Ser or Thr; Xaa_7 is Asp or Glu; Xaa_8 is Leu, Ile, Val, pentylglycine or Met; Xaa_9 is Leu, Ile, pentylglycine, Val or Met; Xaa_{10} is Phe, Tyr or naphthalanine; Xaa_{11} is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa_{12} is Glu or Asp; Xaa_{13} is Trp, Phe, Tyr, or naphthylalanine; Xaa, Xaa_{15}, Xaa_{16} and Xaa_{17} are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa_{18} is Ser, Thr or Tyr; and Z is -OH or $-NH_2$; with the proviso that the

compound does not have the formula of either SEQ. ID. NOS. 1 or 2. Also useful in the present invention are pharmaceutically acceptable salts of the compounds of formula (I).

In one embodiment, the methods of the present invention are directed to reducing gastric motility. In another embodiment, the invention is directed to methods of delaying gastric emptying.

These methods may be used on a subject undergoing a gastrointestinal diagnostic procedure, for example radiological examination or magnetic resonance imaging. Alternatively, these methods may be used to reduce gastric motility in a subject suffering from a gastro-intestinal disorder, for example, spasm (which may be associated with acute diverticulitis, a disorder of the biliary tract or a disorder of the Sphincter of Oddi).

In another aspect, the invention is directed to a method of treating post-prandial dumping syndrome in a subject by administering to the subject a therapeutically effective amount of an exendin or exendin agonist.

In yet another aspect, the invention is directed to a method of treating post-prandial hyperglycemia by administering to a subject a therapeutically effective amount of an exendin or exendin agonist. In a preferred embodiment, the post-prandial hyperglycemia is a consequence of Type 2 diabetes mellitus. In other preferred embodiments, the post-prandial hyperglycemia is a consequence of Type 1 diabetes mellitus or impaired glucose tolerance.

In another aspect, a therapeutically effective amount of an amylin agonist is also administered to the subject. In a preferred aspect, the amylin agonist is an amylin or

an amylin agonist analog such as ^{25,28,29}Pro-human-amylin. The use of amylin agonists to treat post-prandial hyperglycemia, as well as to beneficially regulate gastrointestinal motility, is described in International Application No. PCT/US94/10225, published March 16, 1995 which has been incorporated by reference herein.

In yet another aspect, a therapeutically effective amount of an insulin or insulin analog is also administered, separately or together with an exendin or exendin agonist, to the subject.

In another aspect, the invention is directed to a method of treating ingestion of a toxin by administering an amount of an exendin or an exendin agonist effective to prevent or reduce passage of stomach contents to the intestines and aspirating the stomach contents.

Definitions

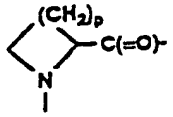
In accordance with the present invention and as used herein, the following terms are defined to have the following meanings, unless explicitly stated otherwise.

The term "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their D and L stereoisomers if their structure allow such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), Lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), typtophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid,

3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisbutyric acid, 2-aminopimelic acid, tertiary-butylglycine, 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylalanine, N-methylglycine, N-methylisoleucine, N-methylpentylglycine, N-methylvaline, naphthalanine, norvaline, norleucine, ornithine, pentylglycine, pipecolic acid and thioproline. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically codified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The term "amino acid residue" refers to radicals having the structure: (1) -C(O)-R-NH-, wherein R typically is -CH(R')-, wherein R' is an amino acid side chain, typically H or a carbon containing substitutent;



or (2) , wherein p is 1, 2 or 3 representing the azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

The term "lower" referred to herein in connection with organic radicals such as alkyl groups defines such groups with up to and including about 6, preferably up to and including 4 and advantageously one or two carbon atoms. Such groups may be straight chain or branched chain.

"Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

In addition, the following abbreviations stand for the following:

"ACN" or "CH_3CN" refers to acetonitrile.

"Boc", "tBoc" or "Tboc" refers to t-butoxy carbonyl.

"DCC" refers to N,N'-dicyclohexylcarbodiimide.

"Fmoc" refers to fluorenylmethoxycarbonyl.

"HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexaflurophosphate.

"HOBt" refers to 1-hydroxybenzotriazole monohydrate.

"homoP" or hPro" refers to homoproline.

"MeAla" or "Nme" refers to N-methylalanine.

"naph" refers to naphthylalanine.

"pG" or pGl"y" refers to pentylglycine.

"tBuG" refers to tertiary-butylglycine.

"ThioP" or tPro" refers to thioproline.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a comparison of the amino acid sequences of exendin 3, exendin 4, and exendin[9-39] using standard single letter rather than three letter amino acid codes.

FIGURE 2 shows GLP-1[7-36]NH_2, exendin-3 and exendin-4 dose-response effects of prior subcutaneous injection on the retention of gastric contents 20 minutes after gavage in normal rats (n = 3-17 for each point). Symbols are means ± SEM and the curves define the best fitting logistic functions. "Zero" indicates the fraction of gastric contents retained in untreated normal rats.

FIGURE 3 shows the dose response effects of prior injection of exendin-4 (n = 29), exendin-4 acid (n = 36) and ^{14}Leu,^{25}Phe exendin-4 (n = 36) on the retention of gastric contents 20 minutes after gavage in normal rats. Symbols are means plus or minus standard error of the mean and the curves define the best fitting logistic functions. "Zero" indicates the fraction of gastric contents retained in untreated normal rats.

FIGURE 4 shows the effect of prior injection of 1.0 μg exendin-4 (sc), n=6; 1.0 μg exendin-4 (sc) plus 0.3 mg exendin[9-39] (sc), n=6; and 0.3 mg exendin[9-39] (sc), n=6 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 4, exendin-4 alone potently inhibited gastric emptying. Exendin[9-39] (sc) alone had no effect on gastric emptying. When injected along with exendin-4, exendin[9-39] did not antagonize the effect of exendin-4 on gastric emptying inhibition.

FIGURE 5 shows the effect of prior injection of 0.3 μg exendin-4 (sc), n=5 and 0.3 μg exendin-4 (sc) plus 0.5 mg exendin[9-39] (iv), n=5 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t+0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 5, exendin-4 alone potently inhibited gastric emptying. When injected along with exendin-4, exendin[9-39] (iv) did not antagonize the effect of exendin-4 on gastric emptying inhibition.

FIGURE 6 shows the effect of prior injection of 10 μg GLP-1[7-36]NH_2 (sc), n=8; 10 μg GLP-1[7-36]NH_2 (sc) plus 3 mg exendin[9-39] (sc), n=6; and 0.3 mg exendin[9-39] (sc), n=6 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 6, GLP-1]7-36]NH_2 potently inhibited gastric emptying. Exendin[9-39] (sc) alone had no effect on gastric emptying. When injected along with GLP-1[7-36]NH_2, exendin[9-39] did not antagonize the effect of GLP-1[7-36]NH_2 on gastric emptying inhibition.

FIGURE 7 shows the effect of prior injection of 10 μg GLP-1[7-36]NH_2 (sc), n=8, and 10 μg GLP-1[7-36]NH_2 (sc) plus 0.5 mg exendin[9-39] (iv), n=3 on the retention of gastric contents 20 minutes after gavage. Also shown are saline controls at t=0 and t=20 min. The error bars show standard error of the mean. As shown in FIGURE 7, GLP-1[7-36]NH_2 alone potently inhibited gastric emptying. When injected along with GLP-1[7-36]NH_2, exendin[9-39] (iv) did not antagonize the effect of GLP-1[7-36]NH_2 on gastric emptying inhibition.

FIGURE 8-1 and 8-2 depicts the amino acid sequences for certain exendin agonists [SEQ. ID. NOS. 5 TO 35].

DETAILED DESCRIPTION OF THE INVENTION

Exendins and exendin agonists (including exendin analogs and exendin derivatives) are useful in this invention in view of their pharmacological properties. Activity as exendin agonists can be indicated by activity in the assays described below. Effects of exendins or exendin agonists on gastric motility and gastric emptying can be identified, evaluated, or screened for, using the methods described in Examples 1-3 below, or other art-known or equivalent methods for determining gastric motility. Negative receptor assays or screens for exendin agonist compounds or candidate exendin agonist compounds, such as a GLP-1 receptor preparation, an amylin receptor assay/screen using an amylin receptor preparation as described in U.S. Patent No. 5,264,372, issued November 23, 1993, the contents of which are incorporated herein by reference, one or more calcitonin receptor assays/screens using, for example, T47D and MCF7 breast carcinoma cells, which contain calcium receptors coupled to the stimulation of adenyl cyclase activity, and/or a CGRP receptor assay/screen using, for example, SK-N-MC cells, can be used to evaluate and/or confirm exendin agonist activity.

One such method for use in identifying or evaluating the ability of a compound to slow gastric motility, comprises: (a) bringing together a test sample and a test system, said test sample comprising one or more test compounds, said test system comprising a system for evaluating gastric motility, said system being characterized in that it exhibits, for example, elevated

plasma glucose in response to the introduction to said system of glucose or a meal; and, (b) determining the presence or amount of a rise in plasma glucose in said system. Positive and/or negative controls may be used as well.

Exendins and exendin agonist compounds such as exendin analogs and exendin derivatives, described herein may be prepared through peptide purification as described in, for example, Eng, et al., J. Biol. Chem. 265:20259-62, 1990; and Eng, et al., J. Biol. Chem. 267:7402-05, 1992, hereby incorporated by reference herein. Alternatively, exendins and exendin agonist peptides may be prepared by methods known to those skilled in the art, for example, as described in Raufman, et al. (J. Biol. Chem. 267:21432-37, 1992), hereby incorporated by reference herein, using standard solid-phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. Typically, an α-N-carbamoyl protected amino acid and an amino acid attached to the growing peptide chain on a resin are coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidinone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the presence of a base such as diisopropylethylamine. The α-N-carbamoyl protecting group is removed from the resulting peptide-resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired N-protected amino acid to be added to the peptide chain. Suitable N-protecting groups are well known in the art, with t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbonyl

(Fmoc) being preferred herein.

The solvents, amino acid derivatives and 4-methylbenzhydryl-amine resin used in the peptide synthesizer may be purchased from Applied Biosystems Inc. (Foster City, CA). The side-chain protected amino acids, such as Boc-Arg(Mts), Fmoc-Arg(Pmc), Boc-Thr(Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl), Fmoc-Ser(t-Bu), Boc-Tyr(BrZ), Fmoc-Tyr(t-Bu), Boc-Lys(Cl-Z), Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-His(Trt), Fmoc-Asn(Trt), and Fmoc-Gln(Trt) may be purchased from Applied Biosystems, Inc. Boc-His(BOM) may be purchased from Applied Biosystems, Inc. or Bachem Inc. (Torrance, CA). Anisole, methylsulfide, phenol, ethanedithiol, and thioanisole may be obtained from Aldrich Chemical Company (Milwaukee, WI). Air Products and Chemicals (Allentown, PA) supplies HF. Ethyl ether, acetic acid and methanol may be purchased from Fisher Scientific (Pittsburgh, PA).

Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer (Model 430A, Applied Biosystems Inc., Foster City, CA) using the NMP/HOBt (Option 1) system and tBoc or Fmoc chemistry (see, Applied Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, CA) with capping. Boc-peptide-resins may be cleaved with HF (-5°C to 0°C, 1 hour). The peptide may be extracted from the resin with alternating water and acetic acid, and the filtrates lyophilized. The Fmoc-peptide resins may be cleaved according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc., 1990, pp. 6-12). Peptides may be also assembled using an Advanced

Chem Tech Synthesizer (Model MPS 350, Louisville, Kentucky). Peptides may be purified by RP-HPLC (preparative and analytical) using a Waters Delta Prep 3000 system. A C4, C8 or C18 preparative column (10 μ, 2.2 x 25 cm; Vydac, Hesperia, CA) may be used to isolate peptides, and purity may be determined using a C4, C8 or C18 analytical column (5 μ, 0.46 x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1% TFA/CH_3CN) may be delivered to the analytical column at a flowrate of 1.0 ml/min and to the preparative column at 15 ml/min. Amino acid analyses may be performed on the Waters Pico Tag system and processed using the Maxima program. The peptides may be hydrolyzed by vapor-phase acid hydrolysis (115°C, 20-24 h). Hydrolysates may be derivatized and analyzed by standard methods (Cohen, S.A., Meys, M., and Tarrin, T.L. (1989), The Pico Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis, pp. 11-52, Millipore Corporation, Milford, MA). Fast atom bombardment analysis may be carried out by M-Scan, Incorporated (West Chester, PA). Mass calibration may be performed using cesium iodide or cesium iodide/glycerol. Plasma desorption ionization analysis using time of flight detection may be carried out on an Applied Biosystems Bio-Ion 20 mass spectrometer.

Peptide compounds useful in the invention may also be prepared using recombinant DNA techniques, using methods now known in the art. See, e.g., Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor (1989). Alternatively, such compounds may be prepared by homogeneous phase peptide synthesis methods.

The use of exendin analogs or derivatives is included within the methods of the present invention. Exendin analogs or derivatives are functional variants having similar amino acid sequence and retaining, to some extent, at least the gastric motility- and gastric emptying-related activities of the related exendin. By "functional variant" is meant an analog or derivative which has an activity that can be substituted for one or more activities of a particular exendin. Preferred functional variants retain all of the activities of a particular exendin, however, the functional variant may have an activity that, when measured quantitatively, is stronger or weaker, as measured in exendin functional assays, for example, such as those disclosed herein. Preferred functional variants have activities that are within about 1% to about 10,000% of the activity of the related exendin, more preferably between about 10% to about 1000%, and more preferably within about 50% to about 500%. Derivatives have at least about 15% sequence similarity, preferably about 70%, more preferably about 90%, and even more preferably about 95% sequence similarity to the related exendin. "Sequence similarity" refers to "homology" observed between amino acid sequences in two different polypeptides, irrespective of polypeptide origin.

The ability of the analog or derivative to retain some activity can be measured using techniques described herein.

Derivatives include modification occurring during or after translation, for example, by phosphorylation, glycosylation, crosslinking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane

molecule or other ligand (see Ferguson *et al.*, Annu. Rev. Biochem. 57:285-320, 1988).

Specific types of analogs include amino acid alterations such as deletions, substitutions, additions, and amino acid modifications. A "deletion" refers to the absence of one or more amino acid residue(s) in the related polypeptide. An "addition" refers to the presence of one or more amino acid residue(s) in the related polypeptide. Additions and deletions to a polypeptide may be at the amino terminus, the carboxy terminus, and/or internal. Amino acid "modification" refers to the alteration of a naturally occurring amino acid to produce a non-naturally occurring amino acid. A "substitution" refers to the replacement of one or more amino acid residue(s) by another amino acid residue(s) in the polypeptide. Analogs can contain different combinations of alterations including more than one alteration and different types of alterations.

Preferred analogs have one or more amino acid alteration(s) which do not significantly affect exendin agonist activity. In regions of the exendin not necessary for exendin agonist activity, amino acids may be deleted, added or substituted with less risk of affecting activity. In regions required for exendin agonist activity, amino acid alterations are less preferred as there is a greater risk of affecting exendin activity. Such alterations should be conservative alterations. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional variant.

Conserved regions tend to be more important for protein activity than non-conserved regions. Known

procedures may be used to determine the conserved and non-conserved regions important of receptor activity using *in vitro* mutagenesis techniques or deletion analyses and measuring receptor activity as described by the present disclosure.

Modifications to a specific polypeptide may be deliberate, as through site-directed mutagenesis and amino acid substitution during solid-phase synthesis, or may be accidental such as through mutations in hosts or systems which produce the polypeptide.

Compounds particularly useful according to the present invention are exendin agonist compounds of the formula (I) [SEQ. ID. NO. 4]:

1 5 10
Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_4 Xaa_5 Xaa_6 Xaa_7 Xaa_8
15 20
Ser Lys Gln Xaa_9 Glu Glu Glu Ala Val Arg Leu

25 30
Xaa_{10} Xaa_{11} Xaa_{12} Xaa_{13} Leu Lys Asn Gly Gly Xaa_{14}
35
Ser Ser Gly Ala Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18}-Z

wherein Xaa_1 is His, Arg or Tyr; Xaa_2 is Ser, Gly, Ala or Thr; Xaa_3 is Asp or Glu; Xaa is Phe, Tyr or naphthalanine;Xaa_5 is Thr or Ser; Xaa_6 is Ser or Thr; Xaa_7 is Asp or Glu; Xaa_8 is Leu, Ile, Val, pentylglycine or Met; Xaa_9 is Leu, Ile, pentylglycine, Val or Met; Xaa_{10} is Phe, Tyr or naphthalanine; Xaa_{11} is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa_{12} is Glu or Asp; Xaa_{13} is Trp, Phe, Tyr, or naphthylalanine; Xaa_{14}, Xaa_{15}, Xaa_{16} and Xaa_{17} are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa_{18} is Ser, Thr or Tyr; and Z is -OH or $-NH_2$; with the proviso that the

compound does not have the formula of either SEQ. ID. NOS. 1 or 2. Preferred N-alkyl groups for N-alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms. Suitable compounds include those having amino acid sequences of SEQ. ID. NOS. 5 to 35.

Preferred exendin agonist compounds include those wherein Xaa_1 is His or Tyr. More preferably Xaa_1 is His.

Preferred are those compounds wherein Xaa_2 is Gly.

Preferred are those compounds wherein Xaa_9 is Leu, pentylglycine or Met.

Preferred compounds include those wherein Xaa_{13} is Trp or Phe.

Also preferred are compounds where Xaa_4 is Phe or naphthalanine; Xaa_{11} is Ile or Val and Xaa_{14} Xaa_{15} $Xaa_{16}$and Xaa_{17} are independently selected from Pro, homoproline, thioproline or N-alkylalanine. Preferably N-alkylalanine has a N-alkyl group of 1 to about 6 carbon atoms.

According to an especially preferred aspect, Xaa_{15}, Xaa_{16} and Xaa_{17} are the same amino acid reside.

Preferred are compounds wherein Xaa_{18} is Ser or Tyr, more preferably Ser.

Preferably Z is $-NH_2$.

According to one aspect, preferred are compounds of formula (I) wherein Xaa_1 is His or Tyr, more preferably His; Xaa_2 is Gly; Xaa_4 is Phe or naphthalanine; Xaa_9 is Leu, pentylglycine or Met; Xaa_{10} is Phe or naphthalanine; Xaa_{11} is Ile or Val; Xaa_{14}, Xaa_{15}, Xaa_{16} and Xaa_{17} are independently selected from Pro, homoproline, thioproline or N-alkylalanine; and Xaa_{18} is Ser or Tyr, more preferably Ser. More preferably Z is $-NH_2$.

According to an especially preferred aspect, especially preferred compounds include those of formula (I) wherein: Xaa_1 is His or Arg; Xaa_2 is Gly; Xaa_3 is Asp or Glu; Xaa_4 is Phe or napthylalanine; Xaa_5 is Thr or Ser; Xaa_6 is Ser or Thr; Xaa_7 is Asp or Glu; Xaa_8 is Leu or pentylglycine; Xaa_9 is Leu or pentylglycine; Xaa_{10} is Phe or naphthylalanine; Xaa_{11} is Ile, Val or t-butyltylglycine; Xaa_{12} is Glu or Asp; Xaa_{13} is Trp or Phe; Xaa_{14}, Xaa_{15}, Xaa_{16}, and Xaa_{17} are independently Pro, homoproline, thioproline, or N-methylalanine; Xaa_{18} is Ser or Tyr: and Z is -OH or $-NH_2$; with the proviso that the compound does not have the formula of either SEQ. ID. NOS. 1 or 2. More preferably Z is $-NH_2$. Especially preferred compounds include those having the amino acid sequence of SEQ. ID. NOS. 5, 6, 17, 18, 19, 22, 24, 31, 32 and 35.

According to an especially preferred aspect, provided are compounds where Xaa_9 is Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa_{13} is Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds are believed to exhibit advantageous duration of action and to be less subject to oxidative degration, both in vitro and in vivo, as well as during synthesis of the compound.

The compounds referenced above form salts with various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H_2SO_4, H_3PO_4, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid and camphorsulfonic acid. Salts prepared with bases include ammonium salts, alkali metal salts, e.g. sodium and potassium salts, and alkali earth salts, e.g. calcium and

magnesium salts. Acetate, hydrochloride, and trifluoroacetate salts are preferred. The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

The compounds referenced above form salts with various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H_2SO_4, H_3PO_4, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid and camphorsulfonic acid. Salts prepared with bases include ammonium salts, alkali metal salts, e.g. sodium and potassium salts, and alkali earth salts, e.g. calcium and magnesium salts. Acetate, hydrochloride, and trifluoroacetate salts are preferred. The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

The compounds described above are useful in view of their pharmacological properties. In particular, the compounds of the invention possess activity as agents to regulate gastric motility and to slow gastric emptying, as evidenced by the ability to inhibit gastric emptying

levels in mammals.

As described in Example 1, gastric emptying was measured in normal Sprague Dawley rats using the retention of an acaloric methylcellulose gel containing Phenol Red delivered by gavage. Dye content in stomachs removed after sacrifice 20 minutes later was determined spectroscopically, and was compared to that in rats sacrificed immediately after gavage to assess emptying. The exendins, exendin 3 and exendin 4, dose-dependently inhibited gastric emptying. The ED_{50} of the response to exendin 3 and exendin 4 was 0.1 and 0.08 μg, respectively, demonstrating that the exendins were ~170-290 times more potent than GLP-1[7-36]NH_2 in inhibiting gastric emptying.

As described in Example 2, the effects of exendin-4 and the exendin-4 analogs, exendin-4 acid and ^{14}Leu,^{25}Phe exendin-4, on inhibition of gastric emptying were examined. Exendin-4 and the exendin-4 analogs dose dependently inhibiting gastric emptying. The ED_{50} of exendin-4 was 0.27 μg. The ED_{50}s of exendin-4 acid and ^{14}Leu,^{25}Phe exendin-4 were 0.12 μg and 0.29 μg, respectively, indicating that the potency of the analogs was comparable to that of exendin-4.

As described in Example 3, the effects of exendin-4 and the cloned GLP-1 receptor antagonist, exendin[9-39] on gastric emptying were examined. After 20 minutes, the animals treated with exendin-4 showed potent inhibition of gastric emptying, which was not reversed by exendin[9-39]. This occurred regardless of whether the exendin[9-39] was administered sc or iv. Exendin[9-39] alone had no effect on gastric emptying.

As noted above, exendin[9-39] is a potent antagonist of GLP-1 which binds at the cloned GLP-1

receptor (Fehmann HC, et al., Peptides 15(3): 453-6, 1994; Thorens B, et al., Diabetes 42(11): 1678-82, 1993). Surprisingly, however, exendin[9-39] did not block the effect of exendin-4 on gastric emptying (see Figures 4 and 5). These results indicate that the effects of exendins and exendin agonists on gastric emptying are not due binding of the exendins at the cloned GLP-1 receptor, but instead that the gastric emptying effects of exendins and exendin agonists are due to their action on a separate receptor.

That exendins can act via mechanisms other than those attributable to the cloned GLP-1 receptor is further evidenced by the reported absence of effect of exendin-4 on inhibition of pentagastrin-induced gastric acid secretion, despite the inhibitory effect of GLP-1 on such secretion. Gedulin, et al., Diabetologia, 40(Suppl. 1):A300 (Abstract 1181) (1997). Additionally, as described in commonly assigned U.S. Provisional Patent Application Serial No. 60/034,905, entitled, "Use of Exendins and Agonists Therefor for the Reduction of Food Intake," filed January 7, 1997, peripherally injected exendin inhibited food intake in mice, an action not observed with GLP-1.

Compositions useful in the invention may conveniently be provided in the form of formulations suitable for parenteral (including intravenous, intramuscular and subcutaneous) or nasal or oral administration. In some cases, it will be convenient to provide an exendin or exendin agonist and another anti-emptying agent, such as glucagon, or amylin, or an amylin agonist, in a single composition or solution for administration together. In other cases, it may be more advantageous to administer

another anti-emptying agent separately from said exendin or exendin agonist. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., Remington's Pharmaceutical Sciences by E.W. Martin. See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988).

Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. They can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 5.6 to 7.4. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example, sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate,

propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

The claimed compositions can also be formulated as pharmaceutically acceptable salts (*e.g.*, acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid. Such salts may be prepared by, for example, reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or

by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, or transmucosally.

If desired, solutions of the above compositions may be thickened with a thickening agent such as methyl cellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic surfactant (such as a Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

Compositions useful in the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity.

For use by the physician, the compositions will be provided in dosage unit form containing an amount of an exendin or exendin agonist, for example, exendin 3,

exendin 4, with or without another antiemptying agent. Therapeutically effective amounts of an exendin or exendin agonist for use in the control of gastric emptying and in conditions in which gastric emptying is beneficially slowed or regulated are those that decrease post-prandial blood glucose levels, preferably to no more than about 8 or 9 mM or such that blood glucose levels are reduced as desired. In diabetic or glucose intolerant individuals, plasma glucose levels are higher than in normal individuals. In such individuals, beneficial reduction or "smoothing" of post-prandial blood glucose levels, may be obtained. As will be recognized by those in the field, an effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the patient's physical condition, the blood sugar level or level of inhibition of gastric emptying to be obtained, and other factors.

Such pharmaceutical compositions are useful in causing gastric hypomotility in a subject and may be used as well in other disorders where gastric motility is beneficially reduced.

The effective daily anti-emptying dose of the compounds will typically be in the range of 0.001 or 0.003 to about 5 mg/day, preferably about 0.001 or 0.05 to 2 mg/day and more preferably about 0.001 or 0.01 to 1 mg/day, for a 70 kg patient, administered in a single or divided doses. The exact dose to be administered is determined by the attending clinician and is dependent upon where the particular compound lies within the above quoted range, as well as upon the age, weight and condition of the individual. Administration should begin at the first sign of symptoms or shortly after diagnosis

of diabetes mellitus. Administration may be by injection, preferably subcutaneous or intramuscular. Orally active compounds may be taken orally, however dosages should be increased 5-10 fold.

Generally, in treating or preventing elevated, inappropriate, or undesired post-prandial blood glucose levels, the compounds of this invention may be administered to patients in need of such treatment in a dosage ranges similar to those given above, however, the compounds are administered more frequently, for example, one, two, or three times a day.

The optimal formulation and mode of administration of compounds of the present application to a patient depend on factors known in the art such as the particular disease or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry, and sports animals and pets such as horses, dogs and cats.

To assist in understanding the present invention, the following Examples are included. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

EXAMPLE 1

The following study was carried out to examine the effects of exendin-3 and exendin-4 on gastric emptying and

to compare the effects with GLP-1[7-36]NH_2 treatment in rats. This experiment followed a modification of the method of Scarpignato, et al., Arch. Int. Pharmacodyn. Ther. 246:286-94 (1980).

Male Harlan Sprague Dawley (HSD) rats were used. All animals were housed at 22.7±0.8 C in a 12:12 hour light:dark cycle (experiments being performed during the light cycle) and were fed and watered *ad libitum* (Diet LM-485, Teklad, Madison, WI). Exendin-3 and exendin-4 were synthesized according to standard peptide synthesis methods.

The determination of gastric emptying by the method described below was performed after a fast of ~20 hours to ensure that the stomach contained no chyme that would interfere with spectrophotometric absorbance measurements.

Conscious rats received by gavage, 1.5ml of an acaloric gel containing 1.5% methyl cellulose (M-0262, Sigma Chemical Co, St Louis, MO) and 0.05% phenol red indicator. Twenty minutes after gavage, rats were anesthetized using 5% halothane, the stomach exposed and clamped at the pyloric and lower esophageal sphincters using artery forceps, removed and opened into an alkaline solution which was made up to a fixed volume. Stomach content was derived from the intensity of the phenol red in the alkaline solution, measured by absorbance at a wavelength of 560 nm. In separate experiments on 7 rats, the stomach and small intestine were both excised and opened into an alkaline solution. The quantity of phenol red that could be recovered from the upper gastrointestinal tract within 20 minutes of gavage was 89±4%; dye which appeared to bind irrecoverably to the gut luminal surface may have accounted for the balance. To

account for a maximal dye recovery of less than 100%, percent of stomach contents remaining after 20 min were expressed as a fraction of the gastric contents recovered from control rats sacrificed immediately after gavage in the same experiment. Percent gastric contents remaining = (absorbance at 20 min)/(absorbance at 0 mm) x 100.

In baseline studies, with no drug treatment, gastric emptying over 20 min was determined. In dose-response studies, rats were treated with 0, 0.01, 0.1, 0.3, 1, 5, 10, or 100 μg of exendin 3, exendin 4, or GLP-1(7-36)NH_2. The results are shown in Figure 2. Figure 2 shows that exendins 3 and 4 inhibited gastric emptying with approximately the same ED_{50} of 0.1 μg, whereas GLP-1(7-36)NH_2 has an ED_{50} of approximately 9 μg, indicating that the exendins are ~90 fold more potent than GLP-1 at inhibiting gastric emptying.

As shown in Table I, exendin-3 and exendin-4 were found to be potent inhibitors of gastric emptying. The effect of rat amylin on gastric emptying is also provided as a second positive control and for comparitive purposes.

TABLE I

DOSE μg	GLP-1 (7-36)NH_2		Exendin-3		Exendin-4		Rat Amylin	
	% remaining *(n)	SEM	% remaining *(n)	SEM	% remaining *(n)	SEM	% remaining *(n)	SEM
Saline Control	48.00 (16)	3.50	46.760 (15)	2.360	46.000 (17)	2.000	48.00 (17)	3.5
0.010	no data		58.240 (3)	3.180	no data	2.000	37.60 (2)	2.50
0.100	42.00 (7)	6.50	70.770 (3)	5.600	72.000 (3)	12.000	52.70 (6)	6.30
0.300	29.60 (7)	3.50	86.420 (3)	6.160	98.000 (2)	4.000	88.20 (4)	3.00
1.000	37.20 (9)	2.70	95.330 (3)	0.790	105.000 (1)	0.000	96.80 (9)	2.80
3.000	56.60 (10)	6.10					108.00(4)	2.70
10.000	87.90 (11)	2.70	101.760 (3)	3.390	112.000 (3)	2.000	101.10 (6)	3.60
100.000	103.60 (7)	2.80	103.640 (3)	2.260	103.000 (3)	3.000	101.20 (2)	2.80

*percent of gastric contents remaining 20 minutes after gavage.

EXAMPLE 2

The effects of exendin-4 analogs on inhibition of gastric emptying were examined, and compared to the effects of exendin-4, according to the methods described in Example 1. Male HSD rats were treated with 0.01, 0.1, 0.3, 1, 10 and 100 μg of exendin-4, 0.01, 0.03, 0.1, 1, 10 and 100 μg exendin-4 acid, and 0.1, 0.3, 1, 10 and 100 μg of ^{14}Leu,^{25}Phe exendin-4. Exendin-3, exendin-4 acid and ^{14}Leu,^{25}Phe were synthesized according to standard peptide synthesis methods. The results, shown in Figure 3 and Table II, show that the exendin agonists, exendin-4 acid and ^{14}Leu,^{25}Phe exendin-4, are potent inhibitors of gastric emptying. The EC_{50} of exendin-4 was 0.27 μg. The EC_{50}s of exendin-4 acid and ^{14}Leu,^{25}Phe exendin-4 were comparable (0.12 μg and 0.29 μg, respectively).

TABLE II

Compound	EC_{50}(μg)
exendin-4	0.27
exendin-4 acid	0.12
^{14}Leu,^{25}Phe exendin-4	0.29

EXAMPLE 3

The ability of exendin[9-39], an antagonist of exendin's effects at the cloned GLP-1 receptor, to antagonize the gastric emptying inhibition effect of exendin-4 and GLP-1[7-36]NH_2 was examined according to the methods described in Example 1. Rats were treated with 1.0 μg exendin-4, 1.0 μg exendin-4 with 0.3 mg exendin[9-39], 10 μg GLP-1[7-36]NH_2 10 μg GLP-1[7-36]NH_2 with 0.3 mg exendin[9-39] or with 0.3 mg exendin 9-39 alone. In these studies, exendin[9-39] was give both subcutaneously (sc)

and intravenously (iv). The results of these experiments are shown in Figures 4-7.

As shown in Figures 4 and 5, after 20 minutes, the animals treated with exendin-4 showed extremely potent inhibition of gastric emptying, which was not reversed by exendin[9-39]. This occurred regardless of whether the exendin[9-39] was administered sc or iv. Exendin[9-39] alone had no effect on gastric emptying.

As discussed above, exendin[9-39] is a potent antagonist of GLP-1 which binds at the cloned GLP-1 receptor (Fehmann HC, et al., Peptides 15(3): 453-6, 1994; Thorens B, et al., Diabetes 42(11): 1678-82, 1993). Surprisingly, however, exendin[9-39] did not block the effect of exendin-4 on gastric emptying (see Figures 4 and 5). These results indicate that the effects of exendins on gastric emptying are not due binding of the exendins at the cloned GLP-1 receptor, but instead that the gastric emptying effects of exendins are due to a different receptor.

That exendin[9-39] did not block the effect of GLP-1[7-36]NH_2 on gastric emptying (see Figures 6 and 7) indicates that, in its effects on gastric emptying, GLP-1 is also acting at a receptor other than the cloned GLP-1 receptor (at which exendin[9-39] is a potent antagonist).

EXAMPLE 4

Preparation of amidated peptide having SEQ. ID. NO. [5]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In

general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. However, at some positions coupling was less efficient than expected and double couplings were required. In particular, residues Asp_9, Thr, and Phe_8 all required double coupling. Deprotection (Fmoc group removal) of the growing peptide chain using piperidine was not always efficient. Double deprotection was required at positions Arg_{20}, Val_{19} and Leu_{14}. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 55%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.5 minutes. Electrospray Mass Spectrometry (M): calculated 4131.7; found 4129.3.

EXAMPLE 5

Preparation of Peptide having SEQ. ID. NO. [6]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 25% to 75% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 21.5 minutes. Electrospray Mass Spectrometry (M): calculated 4168.6; found 4171.2.

EXAMPLE 6

Preparation of Peptide having SEQ. ID. NO. [7]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 4147.6; found 4150.2.

EXAMPLE 7

Preparation of Peptide having SEQ. ID. NO. [8]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35% to 65% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.7 minutes. Electrospray Mass Spectrometry (M): calculated 4212.6; found 4213.2.

EXAMPLE 8

Preparation of Peptide having SEQ. ID. NO. [9]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 50% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 16.3 minutes. Electrospray Mass Spectrometry (M): calculated 4262.7; found 4262.4.

EXAMPLE 9

Preparation of Peptide having SEQ. ID. NO. [10]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6

EXAMPLE 10

Preparation of Peptide having SEQ. ID. NO. [11]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4224.7.

EXAMPLE 11

Preparation of Peptide having SEQ. ID. NO. [12]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6

EXAMPLE 12

Preparation of Peptide having SEQ. ID. NO. [13]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4186.6

EXAMPLE 13

Preparation of Peptide having SEQ. ID. NO. [14]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4200.7

EXAMPLE 14

Preparation of Peptide having SEQ. ID. NO. [15]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4200.7

EXAMPLE 15

Preparation of Peptide having SEQ. ID. NO. [16]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4202.7.

EXAMPLE 16

Preparation of Peptide having SEQ. ID. NO. [17]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4145.6.

EXAMPLE 17

Preparation of Peptide having SEQ. ID. NO. [18]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4184.6.

EXAMPLE 18

Preparation of Peptide having SEQ. ID. NO. [19]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4145.6.

EXAMPLE 19

Preparation of Peptide having SEQ. ID. NO. [20]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4224.7.

EXAMPLE 20

Preparation of Peptide having SEQ. ID. NO. [21]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6.

EXAMPLE 21

Preparation of Peptide having SEQ. ID. NO. [22]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4115.5.

EXAMPLE 22

Preparation of Peptide having SEQ. ID. NO. [23]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 4. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4188.6.

EXAMPLE 23

Preparation of Peptide having SEQ. ID. NO. [24]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4131.6.

EXAMPLE 24

Preparation of Peptide having SEQ. ID. NO. [25]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6.

EXAMPLE 25

Preparation of Peptide having SEQ. ID. NO. [26]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4145.6.

EXAMPLE 26

Preparation of Peptide having SEQ. ID. NO. [27]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the thioproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4266.8.

EXAMPLE 27

Preparation of Peptide having SEQ. ID. NO. [28]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the thioproline positions 38, 37 and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4246.8.

EXAMPLE 28

Preparation of Peptide having SEQ. ID. NO. [29]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 4250.8.

EXAMPLE 29

Preparation of Peptide having SEQ. ID. NO. [30]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the homoproline positions 38, 37, and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4234.8.

EXAMPLE 30

Preparation of Peptide having SEQ. ID. NO. [31]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the thioproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30

minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4209.8.

EXAMPLE 31

Preparation of Peptide having SEQ. ID. NO. [32]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4193.7.

EXAMPLE 32

Preparation of Peptide having SEQ. ID. NO. [33]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in

water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3858.2.

EXAMPLE 33

Preparation of Peptide having SEQ. ID. NO. [34]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings are required at the N-methylalanine positions 38, 37 and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3940.3.

EXAMPLE 34

Preparation of Peptide having SEQ. ID. NO. [35]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Additional double couplings

are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3801.1.

EXAMPLE 35

Preparation of C-terminal carboxylic acid Peptides corresponding to the above C-terminal amide sequences.

The above peptides are assembled on the so called Wang resin (p-alkoxybenzylalacohol resin (Bachem, 0.54 mmole/g)) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 1. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry provides an experimentally determined (M).

WE CLAIM:

1. A method of beneficially regulating gastrointestinal motility in a subject comprising administering to said subject a therapeutically effective amount of an exendin or an exendin agonist.

2. A method according to claim 1 wherein said beneficial regulation of gastrointestinal motility comprises reducing gastric motility.

3. A method according to claim 1 wherein said beneficial regulation of gastrointestinal motility comprises delaying gastric emptying.

4. The method according to claim 1, 2 or 3 wherein said exendin is exendin 3.

5. The method according to claim 1, 2 or 3 wherein said exendin agonist is exendin-4.

6. The method according to claim 1, 2 or 3 wherein said subject is undergoing a gastrointestinal diagnostic procedure.

7. The method of claim 6 wherein said gastrointestinal diagnostic procedure is a radiological examination.

8. The method of claim 7 wherein said gastrointestinal diagnostic procedure is magnetic resonance imaging.

9. A method according to claim 1, 2 or 3 wherein said gastric motility is associated with a gastrointestinal disorder.

10. A method according to claim 9 wherein said gastrointestinal disorder is a spasm.

11. A method according to claim 10 wherein said spasm is associated with a disorder selected from the group consisting of acute diverticulitis or a disorder of the biliary tract or a disorder of the Sphincter of Oddi.

12. A method of treating postprandial dumping syndrome in a subject comprising administering to said subject a therapeutic effective amount of an exendin or exendin agonist.

13. A method of treating postprandial hyperglycemia comprising administering a therapeutically effective amount of an exendin or exendin agonist.

14. The method according to claim 13 further comprising administering a therapeutically effective amount of an amylin or an amylin agonist.

15. The method according to claim 14 wherein said amylin agonist is ^{25}Pro,^{28}Pro,^{29}Pro-h-amylin.

16. A method of treating postprandial hyperglycemia which is a consequence of type 2 diabetes mellitus comprising administering a therapeutically

effective amount of an exendin or an exendin agonist.

17. A method of treating type 1 diabetes mellitus comprising administering a therapeutically effective amount of an exendin or an exendin agonist.

18. A method of treating impaired glucose tolerance comprising administering a therapeutically effective amount of an exendin or an exendin agonist.

19. A method of treatment for ingestion of a toxin comprising: (a) administering an amount of an exendin or an exendin agonist effective to prevent or reduce the passage of stomach contents to the intestines; and (b) aspirating the contents of the stomach.

20. The method according to claim 1, 2 or 3 wherein said exendin agonist is selected from a peptide compound of the formula:

1 5 10
Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_4 Xaa_5 Xaa_6 Xaa_7 Xaa_8
15 20
Ser Lys Gln Xaa_9 Glu Glu Glu Ala Val Arg Leu
25 30
Xaa_{10} Xaa_{11} Xaa_{12} Xaa_{13} Leu Lys Asn Gly Gly Xaa_{14}
35
Ser Ser Gly Ala Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18}-Z

wherein Xaa_1 is His, Arg or Tyr;
Xaa_2 is Ser, Gly, Ala or Thr;
Xaa_3 is Asp or Glu;

Xaa_4 is Phe, Tyr or naphthylalanine;
Xaa_5 is Thr or Ser;
Xaa_6 is Ser or Thr;
Xaa_7 is Asp or Glu;
Xaa_8 is Leu, Ile, Val, pentylglycine or Met;
Xaa_9 is Leu, Ile, pentylglycine, Val or Met;
Xaa_{10} is Phe, Tyr or naphthylalanine;
Xaa_{11} is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met;
Xaa_{12} is Glu or Asp;
Xaa_{13} is Trp, Phe, Tyr, or naphthylalanine;
Xaa_{14}, Xaa_{15}, Xaa_{16} and Xaa_{17}, are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine;
Xaa_{18} is Ser, Thr or Tyr; and
Z is -OH or $-NH_2$;
with the proviso that the compound does not have the formula of either exendin-3 [SEQ. ID. NO. 1] or exendin-4 [SEQ. ID. NO. 2] and pharmaceutically acceptable salts thereof.

21. The method according to claim 1, 2 or 3 wherein said exendin agonist is selected from a peptide compound of the formula [SEQ. ID. NO. 36]:

Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_4 Xaa_5 Xaa_6 Xaa_7 Xaa_8
Ser Lys Gln Xaa_9 Glu Glu Glu Ala Val Arg Leu
Xaa_{10} Xaa_{11} Xaa_{12} Xaa_{13} Leu Lys Asn Gly Gly Xaa_{14}
Ser Ser Gly Ala Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18}-Z

wherein Xaa_1 is His or Arg;
Xaa_2 is Ser, Gly;
Xaa_3 is Asp or Glu;
Xaa_4 is Phe or naphthylalanine;
Xaa_5 is Thr or Ser;
Xaa_6 is Ser or Thr;
Xaa_7 is Asp or Glu;
Xaa_8 is Leu or pentylglycine;
Xaa_9 is Leu or pentylglycine;
Xaa_{10} is Phe or naphthylalanine;
Xaa_{11} is Ile, Val or tert-butylglycine;
Xaa_{12} is Glu or Asp;
Xaa_{13} is Trp or Phe;
Xaa_{14}, Xaa_{15}, Xaa_{16} and Xaa_{17} are independently Pro, homoproline, thioproline or N-methylalanine;
Xaa_{18} is Ser or Tyr; and
Z is -OH or $-NH_2$;
with the proviso that the compound does not have the formula of either exendin-3 [SEQ. ID. NO. 1] or exendin-4 [SEQ. ID. NO. 2] and pharmaceutically acceptable salts thereof.

Figure 1

GLP-1 (7-37)
HAEGTFTSDV SSYLEGQAAK EFIAWLVKGR G-NH_2

Exendin-3
HSDGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS-NH_2

Exendin-4
HGEGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS-NH_2

Exendin[9-39]
DL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS-NH_2

FIGURE 2

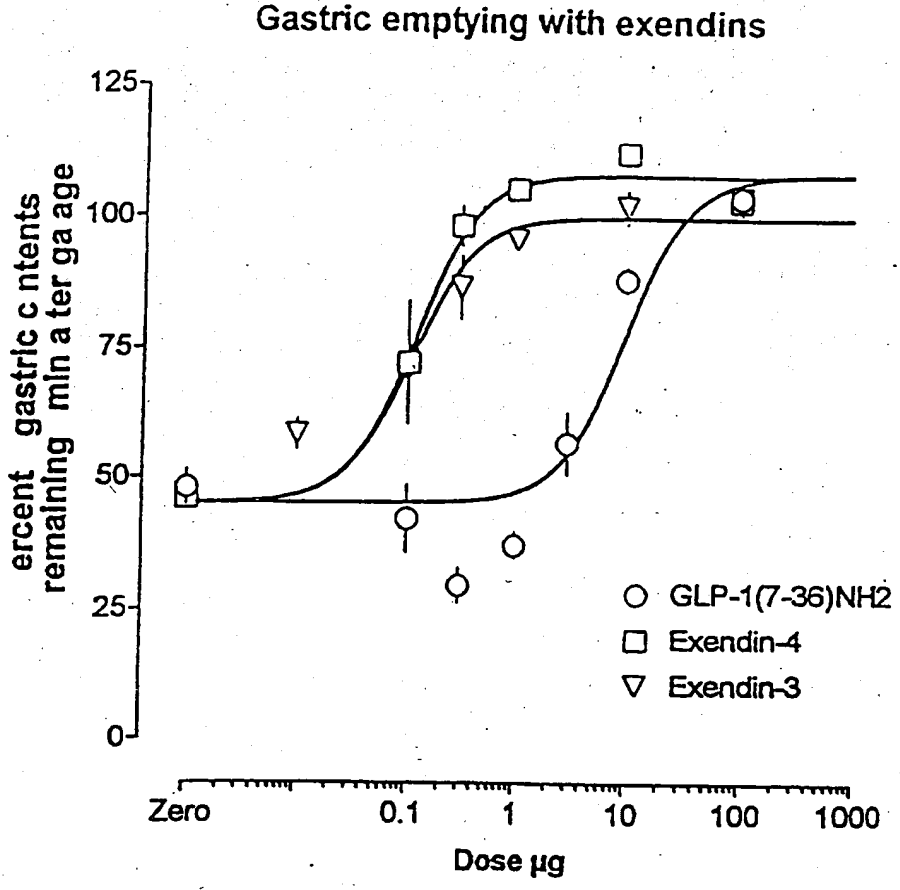

Gastric emptying with exendins
ercent gastric c ntents
remalning mln a ter ga age
125
100
75
50
25
0
Zero
0.1
1
10
100
1000
Dose µg
GLP-1(7-36)NH2
Exendin-4
Exendin-3

Figure 3

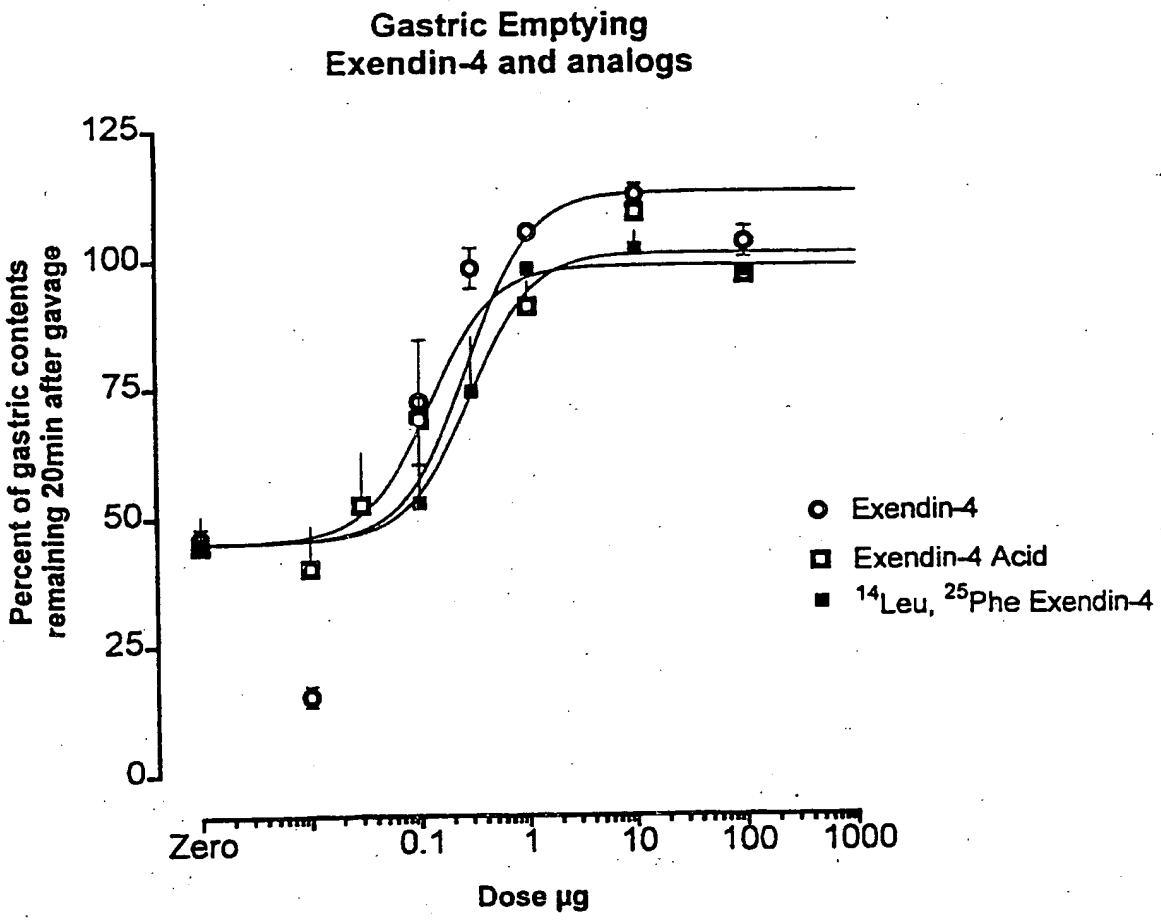

Gastric Emptying
Exendin-4 and analogs
Percent of gastric contents remaining 20min after gavage
125
100
75
50
25
0
Zero
0.1
1
10
100
1000
Dose µg
Exendin-4
Exendin-4 Acid
14Leu, 25Phe Exendin-4

Figure 4

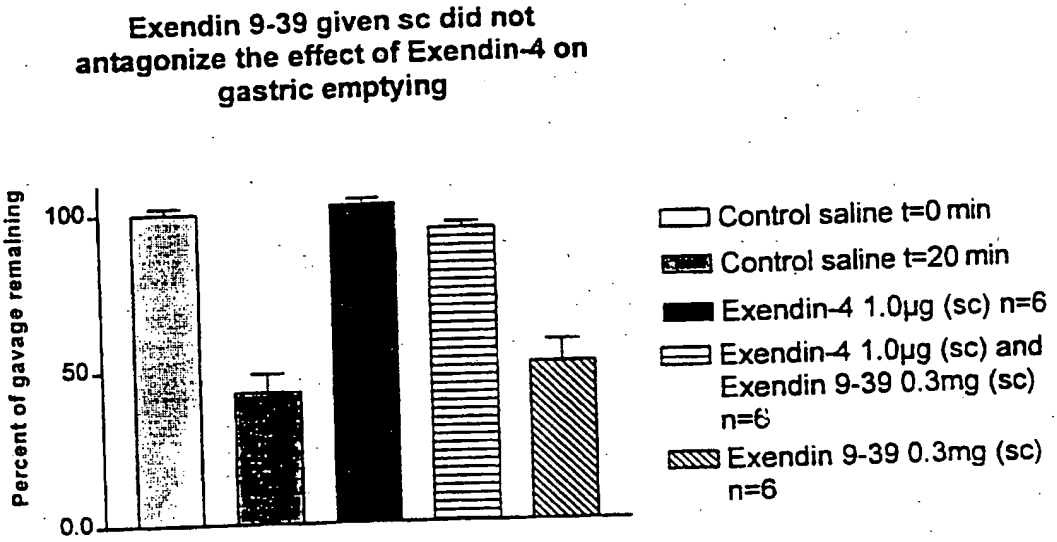

Exendin 9-39 given sc did not antagonize the effect of Exendin-4 on gastric emptying
Percent of gavage remaining
100
50
0.0
Control saline t=0 min
Control saline t=20 min
Exendin-4 1.0µg (sc) n=6
Exendin-4 1.0µg (sc) and Exendin 9-39 0.3mg (sc) n=6
Exendin 9-39 0.3mg (sc) n=6

Figure 5

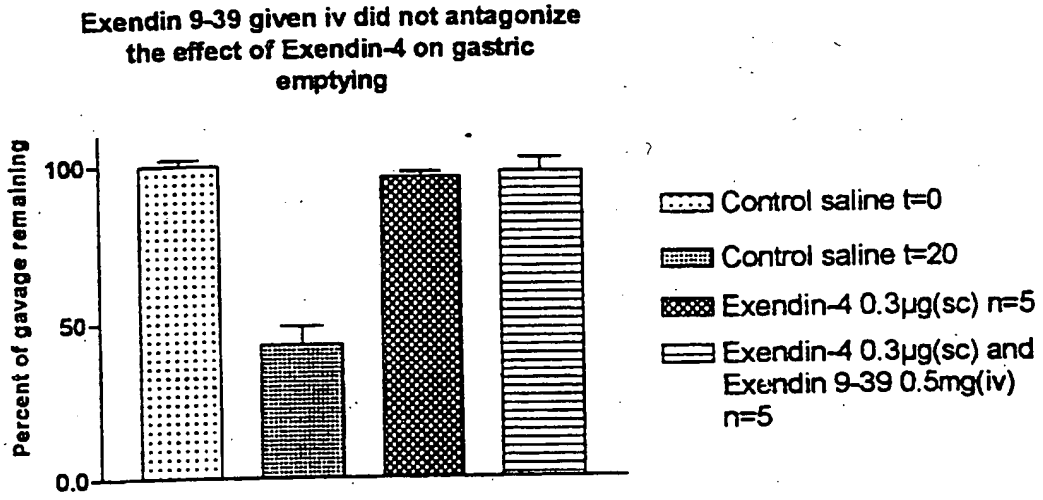

Exendin 9-39 given iv did not antagonize the effect of Exendin-4 on gastric emptying
Percent of gavage remaining
100
50
0.0
Control saline t=0
Control saline t=20
Exendin-4 0.3µg(sc) n=5
Exendin-4 0.3µg(sc) and Exendin 9-39 0.5mg(iv) n=5

Figure 6

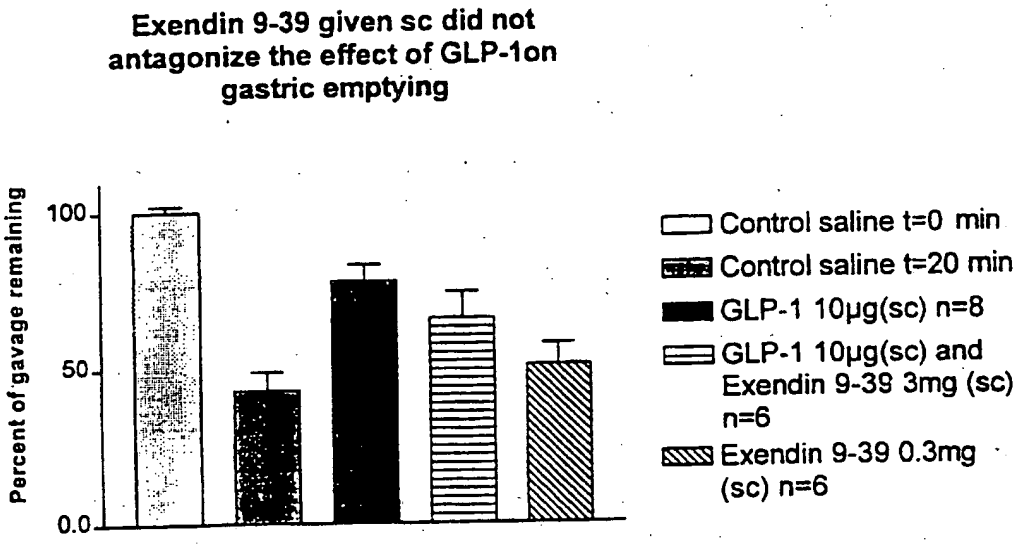

Exendin 9-39 given sc did not antagonize the effect of GLP-1on gastric emptying
Percent of gavage remaining
100
50
0.0
Control saline t=0 min
Control saline t=20 min
GLP-1 10µg(sc) n=8
GLP-1 10µg(sc) and Exendin 9-39 3mg (sc) n=6
Exendin 9-39 0.3mg (sc) n=6

Figure 7

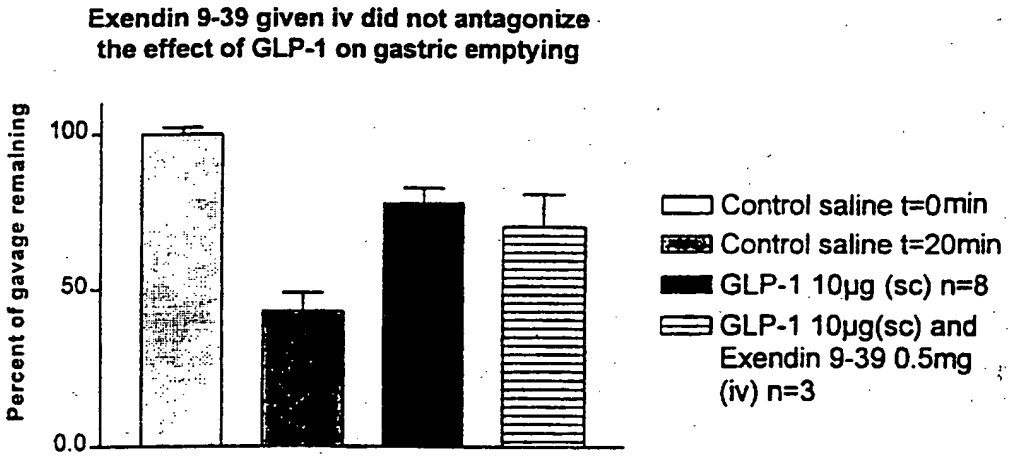

Exendin 9-39 given iv did not antagonize the effect of GLP-1 on gastric emptying
Percent of gavage remaining
100
50
0.0
Control saline t=0min
Control saline t=20min
GLP-1 10µg (sc) n=8
GLP-1 10µg(sc) and Exendin 9-39 0.5mg (iv) n=3

FIGURE 8-1

1 5 10 15 20

Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_6 Xaa_7 Xaa_8 Xaa_9 Xaa_{10} Ser Lys Gln Xaa_{14} Glu Glu Glu Ala Val Arg Leu

25 30 35

Xaa_{28} Xaa_{29} Xaa_{30} Xaa_{31} Leu Lys Asn Gly Gly Xaa_{36} Ser Ser Gly Ala Xaa_{40} Xaa_{41} Xaa_{42} Xaa_{43}-Z

Compound (SEQ. ID. NO.)	Xaa_1	Xaa_2	Xaa_3	Xaa_6	Xaa_7	Xaa_8	Xaa_9	Xaa_{10}	Xaa_{14}	Xaa_{28}	Xaa_{29}	Xaa_{30}	Xaa_{31}	Xaa_{36}	Xaa_{40}	Xaa_{41}	Xaa_{42}	Xaa_{43}	Z
1 [5]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
2 [6]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
3 [7]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
4 [8]	Tyr	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
5 [9]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Tyr	NH_2
6 [10]	His	Gly	Asp	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
7 [11]	His	Gly	Glu	naph	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
8 [12]	His	Gly	Glu	Phe	Ser	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
9 [13]	His	Gly	Glu	Phe	Ser	Thr	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
10 [14]	His	Gly	Glu	Phe	Thr	Thr	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
11 [15]	His	Gly	Glu	Phe	Thr	Ser	Glu	Leu	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
12 [16]	His	Gly	Glu	Phe	Thr	Ser	Asp	pGly	Met	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
13 [17]	His	Gly	Glu	Phe	Thr	Ser	Asp	pGly	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
14 [18]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	pGly	Phe	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2

FIGURE 8-2

Compound (SEQ. ID. NO.)	Xaa_1	Xaa_2	Xaa_3	Xaa_4	Xaa_5	Xaa_6	Xaa_7	Xaa_8	Xaa_9	Xaa_{10}	Xaa_{11}	Xaa_{12}	Xaa_{13}	Xaa_{14}	Xaa_{15}	Xaa_{16}	Xaa_{17}	Xaa_{18}	Z
15 [19]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	pGly	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
16 [20]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	naph	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
17 [21]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Val	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
18 [22]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Val	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
19 [23]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	tBuG	Glu	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
20 [24]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	tBuG	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
21 [25]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Asp	Trp	Pro	Pro	Pro	Pro	Ser	NH_2
22 [26]	His	Ala	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Ser	NH_2
23 [27]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	tPro	tPro	tPro	tPro	Ser	NH_2
24 [28]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	tPro	Ser	NH_2
25 [29]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	hPro	hPro	hPro	hPro	Ser	NH_2
26 [30]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	hPro	hPro	hPro	Ser	NH_2
27 [31]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	tPro	tPro	tPro	tPro	Ser	NH_2
28 [32]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	hPro	hPro	hPro	hPro	Ser	NH_2
29 [33]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	MeAla	MeAla	MeAla	MeAla	Ser	NH_2
30 [34]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	MeAla	MeAla	MeAla	Ser	NH_2
31 [35]	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	MeAla	MeAla	MeAla	MeAla	Ser	NH_2

SSSD/S4355. V01

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 38/00, 38/26; G03F 5/00; C07K 2/00, 5/00
US CL : 514/2, 12; 430/30; 530/300, 303, 308
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/2, 12; 430/30; 530/300, 303, 308

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Database on Derwent Information Ltd. AN 93-37955. HELLSTROM et al. GLIP (Glucagon-Like Insulinotropic Peptide) Delays Gastric Emptying as Part of its Antidiabetogenic Effect in Non-Insulin Dependent Diabetes Mellitus (NIDDM). Scand. J. Gastroenterol. 1993, Vol 28, Suppl. 197, page 38, see included Abstract.	1-3 1-11
Y	Database on Derwent Information Ltd. AN 95-39473. SCHIRRA et al. Differential effects of subcutaneous GLP-1 on gastric emptying, insulin release and exocrine pancreatic secretion in men. Gastroenterology. 1995, Vol. 108, No. 4, Suppl., A1003, see included Abstract.	1-4 and 6-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 SEPTEMBER 1997	24 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SUSAN UNGAR Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	SCHIRRA et al. Differential effects of subcutaneous GLP-1 on gastric emptying, antroduodenal motility, and pancreatic function in men. Proceedings of the Association of American Physicians. January, 1997, Vol 109, No. 1, pp. 84-97, Abstract.	1-3
Y	RAI et al. Actions of Helodermatidae venom peptides and mammalian glucagon-like peptides on gastric chief cells. Am. Physiol. J. 1993, Vol. 265 pages G118-G125, see especially Abstract and page G118.	1-5
Y	DANIEL et al. Use of Glucagon in the Treatment of Acute Diverticulitis. Br. Med. J. 21 September 1974, Vol. 3, pages 720-722, see especially Abstract and Page 20.	9-11
Y	US 3,862,301 A (CHERNISH et al.) 18 June 1973, see column 1.	1-3, 6-8
A	MIHOLIC et al. Glucagon-like peptide-1 (GLP-1), Entleerung Des Magnersaltzes und das Dumpingsyndrom nach Gastrekkomie: Gastric substitute emptying, GLP-1, and dumping after total gastrectomy. Chirurgisches Forum. 1991, pages 429-232, see included Abstract.	12
X	NAUCK et al. Effects of subcutaneous Glucagon-like Peptide 1 (GLP-1[7-36 amide]) in Patients with Type 2-Diabetes. 1995. Diabetologia. Vol 38, Suppl. 1, page A39, Abstract No. 148, see entire Abstract	13 and 16
X	WO 95/07098 (AMYLIN PHARMACEUTICALS INC.) 16 March 1995, see especially pages 21 and 23.	14 and 15
X	DUPRE et al. Glucagon-Like Peptide I reduces Postprandial Glycemic Excursions in IDDM. Diabetes. June 1995, Vol 44, No. 6, pages 626-630, see Abstract.	17
X	D'ALESSIO et al. Glucagon-like Peptide 1 enhances Glucose Tolerance both by Stimulation of Insulin Release and by Increasing Insulin-Dependent Glucose Disposal. J. Clin. Invest. May 1994, Vol. 93, No. 5, pages 2263-2266, see Abstract.	18
A	BAYER et al. Advances in Poison Management. Clinical Chemistry. 1996, Vol. 42, No. 8(B), pages 1361-1366, see especially page 1365, last paragraph.	19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 20 and 21
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

No meaningful search could be carried out on claims 20 and 21 because sequences in computer readable form were not submitted with the application and further because claim 20 disclosed no SEQ ID NO. that could have been searched had sequences in computer readable form been submitted.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14199

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, EMBASE, BIOSIS, CAPLUS, DRUGU, MEDLINE, SCISEARCH, TOXLINE, TOXLIT, JICST-EPLUS
search terms: postprandial hyperglycemia, glp-1, IDDM, NIDDM, treat, therapy, glucose tolerance, toxin, stomach motility, gastric empty, poison, exendin

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I. Claims 1-11 and 20-21 are drawn to a method of regulating gastrointestinal motility.
Group II. Claim 12 is drawn to a method of treating postprandial dumping syndrome
Group III. Claims 13-16 are drawn to a method of treating post prandial hyperglycemia.
Group IV. Claim 17 is drawn to a method of treating type 1 diabetes mellitus.
Group V. Claim 18 is drawn to a method of treating impaired glucose tolerance.
Group VI. Claim 19 is drawn to a method of treatment for ingestion of a toxin.

The inventions listed as Groups do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the claims of inventions II-VI are drawn to materially distinct methods which differ at least in objectives, method steps, reagents and/or dosages and/or schedules used, response variables, and criteria for success and which do not relate to the special technical of regulating gastrointestinal motility recited in Group I.